REMARKS

Claim 1 as amended specifies that the "donor nucleic acid is between more than 100 and 1,000,000 bases in length." This feature is supported in the specification at ¶ ¶ 0104 to 0106. Claim 9 is amended to specify that the length of the donor nucleic acid is "between more than 100 and about 3,000 bases." Support for this amendment is found in the specification at ¶ ¶ 0104 to 0106. Claims 2, 5, and 10 have been amended to correct clerical errors. Claim 12 is amended to delete extraneous language. Claim 14 is amended to correct the spelling of the term "hexaethyleneglycol." New Claims 24, 25 and 26 are added to recite the language omitted from claims 8, 10 and 12, respectively.

1. Election/Restrictions

Applicants respectfully acknowledge that the restriction is maintained. Applicants respectfully withdraw claim 22 without prejudice.

2. 35 U.S.C. §112, paragraph 2

Claims 2, 4, 8, 10, 12, and 14 stand rejected under 35 U.S.C. §112, paragraph 2 as indefinite. Applicants agree that the term "of" in Claim 2 is supposed to be "or" and thus have amended Claim 2 accordingly. Claim 2 recites "donor nucleic acid is prepared by chemical synthesis or by an amplification method."

The examiner contends that claim 4 would be indefinite because it would not be clear how a double stranded donor nucleic acid can interact by Watson-Crick base pairing with the adapter nucleic acid.

Applicants respectfully traverse this rejection. Where the donor DNA is fully double-stranded, a person having ordinary skill in the art would readily recognize that the two strands of the donor nucleic acid may at least partially separate; thereby making it possible for one of the two strands to interact with the adapter sequence by Watson-Crick base pairing. Furthermore, the donor DNA that interacts with the adapter segment may not be fully double-stranded. For

example, a double stranded DNA (dsDNA) made with a sticky end or a fully dsDNA that has been submitted to partial denaturation. Accordingly, applicants respectfully request withdrawal of the rejection of claim 2.

Claims 8 and 10 stand rejected. The examiner contends that the word "preferably" renders the claim indefinite. Applicants have amended claims 8 and 10 to omit the word "preferably" and the phrase following the word. Applicants have added new claims 24 and 25 to recite the subject matter omitted from claims 8 and 10, respectfully.

Claim 12 stands rejected as indefinite based upon use of the word "optionally." Applicants have deleted the phrase "optionally interrupted or substituted by one or more heteroatoms, or heterogroups that comprise at least one of these heteroatoms" from claim 12; and have set forth a new claim 26 reciting "[t]he method according to claim 12, wherein the hydrocarbon skeleton is interrupted and/or substituted by one or more heteroatoms, or heterogroups that comprise at least one of these heteroatoms."

Claim 14 stands rejected as indefinite based upon the word "hexaethyleglycol." Applicants have corrected the spelling of this word to word to read "hexaethyleneglycol."

Accordingly, applicants respectfully request withdrawal of the rejections of claims 2, 4, 8, 10, 12, and 14 under Section 112, second paragraph.

3. **35 U.S.C. §102(b)**

Claims 1, 2, 4, 8-13, 15-21 and 23 stand rejected under 35 U.S.C. § 102(b) as anticipated by Chan, et al, J. Biol. Chem., 274: 11541-11548 (April 1999) ("Chan").

Applicants respectfully traverse this rejection. Applicants respectfully disagree with the characterization of Chan's "polyethyleneglycol-linker, spacer-9" (see Chan, p. 11543, Fig. 1) as an "adapter segment" of the presently claimed invention. Chan discloses a method for effecting homologous recombination based upon oligo-directed triple helix formation, wherein the triple helix-forming oligonucleotide (TFO) segment is covalently attached via a flexible linker to a donor segment. The method of homologous recombination described by Chan uses short donor DNA

fragments that consist of 40-44 nucleotides (see Chan, Abstract, and p. 11543, left column, 5th paragraph), and the target site of the TFO-DD conjugate of Chan is restricted to the vicinity of the triple helix.

By contrast, Claim 1 of the present invention recites an "adapter segment comprising an oligonucleotide sequence able to bind at least a portion of said donor nucleic acid through Watson-Crick base pairing, the adapter segment being linked to said third strand oligonucleotide." The adapter sequence is covalently linked to TFO via a spacer which is comprised of a flexible chain, and is non-covalently linked to the donor nucleic acid through Watson-Crick base pairing (¶0114). The presently claimed invention in claim 1 sets forth a "donor nucleic acid [that] is between more than 100 and 1,000,000 bases in length." The presently claimed invention allows site-directed mutagenesis at a site which is not distance-limited on the triple helix-forming site (¶0081). For example, the target site of the present invention can be hundreds of base pairs away from the triplex site.

Chan does not disclose a TFO and a donor DNA (DD) joined together by non-covalent interaction through an adapter oligonucleotide. Chan also does not disclose a method of homologous recombination using donor DNA fragments that consist of 100-1,000,000 bases in length. Furthermore, Chan does not disclose site-directed mutagenesis at a site which is not distance-limited on the triple helix-forming site. Accordingly, Chan does not anticipate claims 1, 2, 4, 8-13, 15-21, and 23.

4. **35 U.S.C. §103(a)**

Applicants confirm that the subject matter of the various claims of the application, which currently names joint inventors, was commonly owned at the time any inventions covered there in were made.

5. **35 U.S.C. §103(a)**

Claims 1-5, 8-13, 15-21, and 23 stand rejected under 35 U.S.C. §103(a) as obvious over Chan in view of Erdeniz, et al., Genome Research 7: 1774-1183 (1997) ("Erdeniz").

Applicants respectfully traverse this rejection. As noted above, the claimed method allows mutagenesis to take place at a site which is not distance-limited vis-a-vis the triple helix-forming site (¶¶0013, 0081). This result relies first, on the non-covalent interaction between the TFO, which defines the homing site, and the donor DNA. The non-covalent interaction is mediated by the adapter and makes it possible for the donor DNA to dissociate from the TFO/adapter construct and to undergo recombination at a site remote from the homing site of the TFO.

Second, the use of an adapter segment provides a recombination system which is versatile and makes it possible to easily associate the adapter with long donor nucleic acid sequences, and in particular, PCR prepared donor nucleic acids. A mutation may be introduced at any position of the long donor nucleic acid, and recombination may take place at any distance from the TFO homing site (see for example ¶0230).

By contrast, Chan describes a recombination system that uses short donor DNA fragments of 40-44 nucleotides wherein a single point mutation is localized in the center of the donor DNA fragment (see Chan, p. 11544, left column, 1st paragraph). Chan observes that a covalent link between the donor DNA and the TFO make it possible to increase the efficiency of recombination (see Chan, p. 11547, right column, 1st paragraph of the "Discussion" section). Chan further suggests that this apparent synergy may rely on a mechanism of DNA repair that may be induced by triplex formation, at or around the triplex target site (see Chan, p. 11548, left column, 2nd paragraph).

Therefore, a person having ordinary skill in the art would readily infer from Chan that in order to benefit from the induction of DNA repair and to obtain an enhance efficiency of recombination, the recombination site should be localized at or around the TFO homing site. Accordingly, Chan does not provide direction to one having ordinary skill in the art as to how to carry out the presently claimed invention.

Erdeniz describes a method for effecting homologous recombination in which DNA sequences are PCR amplified with a pair of adaptamers to direct fusion of the amplified sequences.

This allele replacement method is restricted to yeast (see Erdeniz, p. 1181, left column, beginning of 2nd paragraph).

Neither Chan nor Erdeniz provide motivation for one skilled in the art to combine the two disclosures to arrive at the claimed invention. Furthermore, the teaching of Erdeniz would not have further helped one skilled in the art to practice the claimed method in view of Chan. Accordingly, applicants respectfully request withdrawal of the rejection of claims 1-5, 8-13, 15-21, and 23 under 35 U.S.C. §103(a).

6. **35 U.S.C. §103(a)**

Claims 1, 2, 4, 8-21 and 23 stand rejected under 35 U.S.C. §103(a) as obvious over Chan in view of U.S. Patent No. 5,770,408 to Sato, et al. ("Sato").

Applicants respectfully traverse this rejection. As noted above, the presently claimed "method for effecting a homologous recombination between a double-stranded native nucleic acid segment in a cell and a donor nucleic acid segment introduced into the cell" advantageously provides site-directed mutagenesis based on oligonucleotide-directed triple helix formation and homologous recombination which has a high efficiency of recombination, and which allows mutagenesis at a site which is not distance-limited with respect to the triple helix-forming site (¶¶ 0013, 0081, 0230). This results in part from the non-covalent interaction between the TFO, which defines the homing site, and the donor DNA. The non-covalent interaction is mediated by the adapter and makes it possible for the donor DNA to dissociate from the TFO/adapter construct and to undergo recombination at a site remote from the homing site of the TFO.

The use of an adapter segment also provides a recombination system which is versatile and makes it possible to easily associate the adapter with long donor nucleic acid sequences, and in particular, PCR-prepared donor nucleic acids. A mutation may be introduced at any position of the long donor nucleic acid, and recombination may take place at any distance from the TFO homing site (see for example ¶0230).

Chan, as discussed above, describes a recombination system that uses short donor DNA fragments of 40-44 nucleotides wherein a single point mutation is localized in the center of the donor DNA fragment. Furthermore, Chan discloses that a covalent link between the donor DNA and the TFO makes it possible to increase the efficiency of recombination. The apparent synergy between the donor DNA and the TFO may rely on a mechanism of DNA repair that may be induced by triplex formation at or around the triplex target site. Accordingly, Chan discloses that in order to benefit from the induction of DNA repair and to obtain an enhance efficiency of recombination, the recombination site should be localized at or around the TFO homing site.

Sato discloses a ligase chain reaction (LCR) based upon a method for the amplification of a base sequence. Sato teaches the use of a hexaethyleneglycol linker.

As explained above, Chan does not provide an incentive to one having skill in the art to develop a method of homologous recombination that uses an adapter segment to allow an indirect non-covalent binding of the donor DNA to the TFO. Furthermore, the teaching of Sato would not have helped one having skill in the art to carry out the claimed method. Accordingly, applicants respectfully request withdrawal of the rejection of claims 1, 2, 4, 8-13, 15,21, and 23 under 35 U.S.C. §103(a).

7. **35 U.S.C. §112, paragraph 1**

Claims 1-21 and 23 stand rejected under 35 U.S.C. §112, first paragraph. The examiner contends that the specification, while being enabling for *in vitro* site directed mutagenesis of a target DNA molecule or site directed mutagenesis of a target DNA molecule *ex vivo* in cultured or isolated cells, it does not reasonably provide enablement for *in vivo* methods of site directed mutagenesis of a target DNA molecule. The examiner further contends that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Applicants respectfully traverse this rejection.

The Examiner notably relies on Puri et al. (2001) ("Puri"). Puri discloses that purine TFOs, for instance, may be subjected to self structure formation rather than triplex formation.

Applicants respectfully point out that this may be a drawback of natural oligonucleotides, such as TFOs containing cytosine. Triplex formation of TFOs containing cytosine may show pH and salt-dependency, which may sometimes be incompatible with the pH and salt conditions in cells.

Puri et al. did not acknowledge several important works showing that the use of a cytosine analogue or a modified sugar-phosphate backbone can efficiently overcome these limitations.

TFOs with such modifications are encompassed by the method according to the invention. The specification of the instant application recites that the oligonucleotides may have a native phosphodiester backbone or other backbone chemical groups, as well as substituted sugar moieties or nucleobases different from adenine, cystonine, guanine, thymine or uracil (see ¶ 0088). The specification further describes the use of TFOs harboring modified nucleotides or having a modified backbone (see ¶ ¶ 0198 and 0244-0247).

In addition, 2-aminopyridine (2AP), has been reported in 1997 as an useful analogue of cytosine in TFOs (Hildbrand et al. (1997) J. Am. Chem. Soc. 119:5499; Cassidy et al. (1997) Nucleic Acids Res. 25:4891). The pKa of 2AP is 6.86 and can be readily protonated at neutral pH due to the polyelectrolyte property of nucleic acids which condense cations including protons (e.g., the local pH is lower than that in the bulk buffer). The positive charges provided by protonated cytosine analogues make an important contribution to triplex stability.

There are other triplex-motifs that do not involve cytosines, and thus are pH-independent. It was further shown that a positively charged sugar-phosphate backbone considerably reduces the salt dependence of TFOs, including Mg^{2+}, and is compatible with the physiological conditions that prevail in cells.

Puri showed that TFOs containing 2'-O-aminoethyl residues retain high potency to form triple helices even at low Mg^{2+} concentration (1 mM), and are much more efficient for cellular responses than unmodified TFOs (see Puri, Abstract).

TFOs containing residues with *N*-(diethylaminoethyl) and *N*-(dimethylaminopropyl) phosphoramidate linkages are other examples of backbone modifications that form a more stable triplex than those formed with unmodified TFO (see Dagle and Weeks (1996) Nucleic Acids Res. 24:2143; Bailey et al. (1998) Nucleic Acids Res 26:4860; Vasquez et al. (2001) J.Biol.Chem. 276:38536). Triple helix formation by TFOs with a cationic backbone is much less dependent on ionic condition, and less prone to form self associated structures than the TFO with a natural backbone. In addition, the oligonucleotides with a full length cationic backbone were shown to be spontaneously taken up into cells without the need for transfection (Michel et al. (2003) Nucleic Acids Res 31:5282).

As regards to Lin et al. (2001) ("Lin"), it should be pointed out that the TFOs used in this work are unmodified oligonucleotides (see figure 1, page 39118).

Therefore, the statements in Puri and Lin are not correct, as they did not consider modified oligonucleotides. There is a large amount of data available to design appropriate TFOs with modified residues having predictable activity in cells *in vivo*.

All the features discussed above support that TFOs with modified oligonucleotides are prone to triplex formation *in vivo*.

Accordingly, Applicants respectfully assert that the Examiner's rejection, and notably, the Examiner's citation to Puri and Lin, as to the unpredictability of the art in general, are ill-founded in view of the knowledge in the art.

Furthermore, Applicants also point out the disclosure of Vasquez et al. 2000, Science 290:530 ("Vasquez"). Vasquez showed that TFOs can induce mutations at the target genomic sites in somatic cells of adult mice. This result indicates that gene targeting via triple helix formation is possible *in vivo*. Accordingly, the method recited in claims 1-21 "for effecting homologous

recombination between a double-stranded native nucleic acid segment in a cell and a donor nucleic acid segment introduced into the cell" and the method recited in claim 23 "for effecting gene alteration or mutation repair at a specific sequence site on a native DNA" is possible *in vivo* by using the disclosure provided in the specification.

Accordingly, Applicants respectfully assert that the claimed method is enabled for effecting homologous recombination *in vivo*.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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